Chickering, Arthur Merton

1927. Spermatogenesis in the Belostomatidae
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Jour. Morph. and Physiol., v. 44, no. 3, Dec. 5

Cytology { germ cells / cytoplasmic inclusions
Belostomatidae
Spermatogenesis
Cytoplasm { chondriosomes / Golgi bodies

The Wistar Institute Press
Philadelphia, Pa., U.S.A.

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Michigan

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Reprinted from the JOURNAL OF MORPHOLOGY AND PHYSIOLOGY
Vol. 44, No. 3, December, 1927

SPERMATOGENESIS IN THE BELOSTOMATIDAE

II. THE CHROMOSOMES AND CYTOPLASMIC INCLUSIONS IN THE MALE GERM CELLS OF BELOSTOMA FLUMINEUM SAY, LETHOCERUS AMERICANUS LEIDY, AND BENACUS GRISEUS SAY [1]

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SEVEN PLATES (ONE HUNDRED AND FIFTY-THREE FIGURES)

AUTHOR'S ABSTRACT

This paper contains a description of the appearance and behavior of the chromatin and chief cytoplasmic inclusions in the male germ cells of three species of Belostomatidae.

There are eight spermatogonial chromosomes in Lethocerus americanus, twenty-four in Belostoma flumineum, twenty-eight in Benacus griseus. An XY-pair of sex chromosomes occurs in each species. These are identifiable at every step in maturation in Lethocerus, but not in the others. A clear case of heteropycnosis occurs in Benacus. Parasynapsis is believed to be the mode of pairing of chromosomes in all three species. The genesis of three large atelomitic ring tetrads is described in Lethocerus.

Chondriosomes are traced from spermatogonia to spermatids without a break in continuity. Spermatogonial chondriosomes are always granular. During the growth period filaments are formed, probably by fusion of granules, and these are sorted out in approximately equal numbers to spermatids. Benacus is especially favorable for studies on chondriosomes. Likewise, Golgi bodies have been traced throughout the process of maturation. They are minute granules in spermatogonia. During the growth period each becomes a flattened plate-like body clearly differentiated into two materials. Dictyosomes are formed by fragmentation in the first prophase, and these are distributed to spermatids where they fuse to form an acroblast in each. Some minor cytoplasmic inclusions are briefly described.

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INTRODUCTION

The Belostomatidae[1] have been studied by two investigators, Montgomery ('01, '06) and Chickering ('16). In the spermatogonia of Belostoma flumineum or B. aurantiacum Montgomery counted twenty-four chromosomes, two of which remained distinct during the resting stages and were designated as chromosome nucleoli. 'Yolk granules' in the cytoplasm and several masses of idioplasmic material he regarded as comparable to the single idiosome of Peripatus (Montgomery, '11). In the synaptic period these masses of idiosomic material appeared as a single body at one pole of the nucleus. Following synapsis, eleven bivalent chromatin masses were identified and two chromosome nucleoli. Thirteen chromosomes appeared in the first meiotic metaphase; the two smallest were regarded as the XY-pair. The first division was regarded as the reduction division. The second division was found to be equational, and in this division the unequal pair separated before the autosomes divided. Thus spermatids receive twelve chromosomes each and do not differ

[1] Since Henking's pioneer work on Pyrrhocoris in 1891, the behavior of the chromatin in the gametogenesis of bugs has been extensively studied. According to the published lists of Harvey ('16, '20) and my own search through the literature, 117 species of Hemiptera-Heteroptera belonging to sixteen families have been studied by twenty-four investigators up to the beginning of 1926. Species in which the cytoplasmic inclusions have been carefully studied are few. For bibliographies see the papers of Wilson, Montgomery, Harvey, and Bowen.

in number, but only in respect to the kind of chromosomes received.

In 1916, I made a preliminary study of Belostoma flumineum, paying special attention to the chromosomes. The spermatogonial number of chromosomes was shown to be twenty-four. Chromosome nucleoli corresponding to the X- and the Y-chromosomes were not found in the resting spermatogonia, nor were these found to be the two smallest in the metaphase groups as stated by Montgomery. Chromatoid bodies were found in the early growth period and followed through an irregular behavior into the spermatids.

Papers in which the whole course of events involving both chromatin and cytoplasmic inclusions from early spermatogonia to spermatozoa are few in number even in the Hemiptera-Heteroptera, which has long been a favorite group for cytological studies. The present paper is the result of an attempt to follow the history of all the important elements of the male cells, from their appearance in early spermatogonia to their position in young sperm, just as the acroblast comes to rest near the base of the nucleus preparatory to the formation of the acrosome. Completion of the study of spermiogenesis in these animals is contemplated in the near future.

It is with a deep sense of gratitude that I take this opportunity to express my indebtedness to Prof. A. Franklin Shull, under whom this work was completed, for kindly help and criticism.

MATERIAL AND TECHNIQUE

Material

The species of Belostomatidae used in this investigation are three in number, Belostoma flumineum Say, Lethocerus americanus Leidy, and Benacus griseus Say. Dr. Roland F. Hussey, of New York University, has very kindly confirmed my identification of these species.

Belostoma is the smallest and most common of our local 'giant water bugs.' It is easily collected in shallow pools,

ponds, and slowly moving streams, where it may be found clinging to aquatic plants, floating sticks, and trash of almost every sort. The adults winter over in mud and débris and resume an active life early in the spring. It is an easy matter to obtain the eggs of this species in plentiful numbers, because of the habit displayed by the male of carrying a mass of from fifty to one hundred on his back, where they are placed by the female shortly after copulation.

Lethocerus is much less common in the vicinity of Ann Arbor, Michigan, where nearly all the recent material has been collected, than is Belostoma. It appears more infrequently than in former years, when it could be found frequently in large numbers flying about electric street lamps.

Benacus is the largest of the Belostomatids and is also the least common in this locality. A sufficient number of these animals has not been obtained to allow the preparation of the germ cells in all the various ways necessary to a complete study. The most critical stages in the history of the Golgi bodies are thus lacking in this form.

In general, the animals have been killed and their gonads fixed as soon as possible following collection, in order to avoid any possible deleterious changes. Animals killed during the last instar often have the whole chromosome history shown in their germ cells.

Technique

This matter has been treated adequately within very recent years by Bowen ('19, '22) and Gatenby in Lee's Vade Mecum ('21). Hence only a brief summary of the methods used will here be given, with frequent references to special phases of the results.

For the fixation of chromatin the best results have been obtained with Bouin's fluid at room temperature. One hour is long enough for the tissues to remain in the fluid, but a longer time does no harm, although, in studying synapsis, greater care may be necessary in timing the treatment in order to get optimum results. Most of the cytoplasmic ele-

ments are destroyed with this fixative, but this is probably a help in the study of chromosomes.

Flemming's strong solution has been an exceedingly useful fluid. As a general fixative it is to be preferred over all others, because, by modifying it in a variety of ways, it can be made to bring out nearly every element in both nucleus and cytosome at least tolerably well. Spermatozoa in various stages of development are well demonstrated with this fluid. Among numerous other fluids, Gilson's has proved useful, especially following a brief treatment.

Although a number of stains have been used in the course of this work, iron hematoxylin, with or without a counterstain, has again proved to be the best for chromatin.

My experience is in agreement with that of Bowen ('19) and Gatenby ('20), both of whom have strongly emphasized the necessity of adopting special methods for the study of chondriosomes and the Golgi apparatus. It is practically hopeless to attempt to follow these elements through the maturation divisions with any great degree of accuracy following ordinary treatment for chromatin. Of the numerous methods used for the study of chondriosomes, the method of Benda as used by Meves and Duesberg ('08) and Bowen ('20, '22) has been the most successful. This method may be made to bring out the chondriosomes in great brilliance. Flemming's fluid without acetic acid, so strongly recommended by Gatenby, has yielded only mediocre results, but by the addition of a few drops of the acid good results have been obtained.

For the study of the Golgi apparatus in these animals the modified Kopsch (Bowen, '22) and the Mann-Kopsch (Lee, '21) method with variations have been the most useful, particularly in tracing the Golgi bodies through the maturation divisions. Silver-impregnation methods have not yielded good results. In all these methods variable factors appear to be present which necessitate more or less blind experimentation in discovering proper technical treatment in each new case.

OBSERVATIONS

I. Chromatin

a. Spermatogonial divisions. There are four large, reniform chromosomes, two very small ones, and eighteen of intermediate sizes (fig. 1) in Belostoma. Attempts to pair the elements according to size are successful for only eight. The small pair was mistakenly identified by Montgomery ('01) as the XY-pair, but he later concluded ('06) that the fourth largest element and the third from the smallest constitute the sex-chromosome pair. This is probably correct, although identification is uncertain at this time.

Spermatogonial metaphase plates in Benacus show twenty-eight chromosomes. No large reniform elements are present, all of them being ovoid or spherical in form (figs. 2, 3) and of much more uniform size than in Belostoma. Attempts to homologize the various pairs of chromosomes have been attended with slight success, although there can be no doubt that such an homology exists here as elsewhere. The first of a series of strong resemblances between Benacus and Pyrrhocoris apterus throughout the history of the chromatin will be seen from a comparison of figures 2 and 3 with Gross' figures 9 and 10. Although superficially the chromosome-complex of Benacus appears very different from that of Belostoma a closer inspection will show that if the four largest chromosomes of the latter were broken up into two equal portions each, a complex would be produced closely resembling that of Benacus. That this has actually occurred can only be conjectured, but, in the light of recent work upon several groups of plants and certain groups of animals as well, the conjecture seems plausible (Painter, '25). Definite identification of the sex chromosomes in the spermatogonia is impossible, but it is certain that they are among those of intermediate size.

The spermatogonial chromosomes of Lethocerus are eight in number (figs. 4, 5, 6). Among these are four reniform bodies, two others which are more or less twisted, and finally two small spherical chromosomes. These last are clearly

identifiable as the XY-pair in nearly all stages throughout spermatogenesis, but no constant size differences exist between them. Four pairs of homologous chromosomes are evident in all clear metaphase plates.

Lethocerus americanus, with eight chromosomes, has the distinction of possessing the next to the smallest number thus far recorded for true bugs, with Pentatoma senilis (Wilson, '13) having the smallest number, six. It is difficult to see just how the chromosome complex of Lethocerus is related to those of the other two species. If we suppose that Lethocerus represents the most primitive form, then by fragmentation of two large chromosomes the numerous smaller elements of Belostoma may have been produced. As already suggested, by further fragmentation the chromosome complex of Benacus may have been produced from a group like that of Belostoma.

Chance seems to determine the position of the individual chromosomes in the metaphase plates. In Lethocerus, where the sex chromosomes can always be identified, these only occasionally lie together (fig. 6). More frequently, they are separated by one or more of the large autosomes (fig. 4). The same is true of the large autosomes of Benacus and Belostoma. Thus no pairing of homologous chromosomes occurs here as appears so strikingly in the Diptera (Stevens, '08; Metz, '14, '16, '22) and also in a few Hemiptera (Montgomery, '06), and in certain other animals and plants.

The earliest spermatogonia are the largest cells in the whole germ line, excepting only the primary spermatocytes. The later cells are much diminished in size, but are more favorable for study, because their chromosomes tend to flatten out more in the metaphase plates. Mitosis is quite regular and without special features except in Benacus, as described later. In Lethocerus the chromosomes can be followed through mitosis with great precision. From an early stage in prophase the spireme appears to be cut up into eight segments, and these are probably split longitudinally when they go upon the spindle.

In all three species certain cells in the walls of follicles and cysts appear regularly tetraploid, and probably show a complete duplication of all pairs of chromosomes. Often, and perhaps always, the tetraploidy is correlated with an increased size of the cells as a whole.

b. Growth period to diakinesis. In all three species the final spermatogonial telophase is regular, with the chromosomes split longitudinally as in normal mitoses. The only irregularity observed is in regard to the behavior of the sex chromosomes in Benacus. In all three forms the autosomes and, in Belostoma and Lethocerus, the sex chromosomes also are reduced to a fairly typical net in the telophase of the last division.

Although evidence was formerly lacking (Chickering, '16) that 'chromosome nucleoli' were present throughout the growth period in Belostoma, it is now certain that this form falls into line with many other Hemiptera in this respect. In this animal the sex chromosomes condense into compact bodies at the initiation of the leptotene stage: in Lethocerus this occurs some time after synapsis; while in Benacus they become visible as compact elements as early as the next to the last spermatogonial anaphase and retain this form throughout the growth period. There is some evidence, not yet conclusive, that the sex chromosomes in Benacus are always distinct in the telophase and resting nuclei of spermatogonia. It is certain, however, that at a late anaphase, probably the last but one, two chromosomes fail to lose their compact form and staining power as do the autosomes (fig. 25). There is a slight difference in the size of these two bodies, and this seems to be characteristic of the XY-pair in this species during the growth period, although during the reduction division no constant differences can be seen. In the telophase of this division the difference between these two chromosomes and the autosomes becomes more marked (figs. 26, 27). They retain their strong affinity for dyes even in the resting condition, and from this time on there is no break in their continuity throughout the maturation process.

Since a prochromosome stage seems to occur regularly in many insects, it has been carefully sought for here. None has been discovered, and thus appears to be absent from Belostomatidae.

It is highly probable that the net-like stage following the last telophase is gradually but directly transformed into a stage showing a mass of exceedingly fine threads running throughout the nucleus in an intricate fashion (fig. 28). This preleptotene stage is quickly organized into the true leptotene in which the threads are more definite. In Lethocerus, which has yielded the clearest preparations, the leptotene stage is preceded by a stage in which the net is in the condition shown in figure 34. This stage reminds one somewhat of the unraveling stage of Oncopeltus and Lygaeus (Wilson, '12). These much-branched threads appear to be directly transformed into the leptotene threads (fig. 35). The extremely fine granular threads are always somewhat withdrawn from the nuclear membrane, thus foreshadowing the marked contraction of the next stage. At no time is there any indication of a polarization of these threads, and this, together with their extreme attenuation, makes counting of them practically impossible. In the latter part of this stage a parallel arrangement of threads can very frequently be found (fig. 35). Its constancy and resemblance to the onset of synapsis in other forms in which there is less obscurity make it probable that this stage is actually the beginning of the pairing process which is accomplished in the next stage. In nuclei which are quite certainly representative of a later stage, however, there appears to be no trace of pairing (fig. 36).

In all cases the leptotene stage is rapidly followed by a pronounced synizesis (figs. 29, 30, 38 to 40). The threads shorten and thicken considerably, and increase their avidity for dyes to such an extent that the knots are often little more than black blotches. These gradually tighten and often take an excentric position (figs. 30, 38, 39). In sufficiently destained preparations individual threads can be seen, but no analysis of their relationship to each other has been possible.

The period of synizesis is followed by a stage in which the nucleus is filled with heavily staining but less compact threads, which usually give no indication of a dual nature (figs. 31, 32, 43, 44). In Benacus and Belostoma these pachytene threads are too numerous and too thoroughly interlaced to allow an accurate counting. But because of the close resemblance in many ways of Benacus to Pyrrhocoris and Leptocoris, in which the number of threads at this stage has been reported as diploid (Gross, '06; Yocom, '23), special attention has been given this point. It is, I believe, certain that the number is much less than diploid. The small number of chromosomes and corresponding threads in Lethocerus make it possible to count the separate elements in this stage with reasonable certainty. Particularly favorable nuclei show the number to be five. Three of these are long much-twisted threads, while the other two are comparatively short and straight rods. It is practically certain that these are the three bivalent and two univalent elements which become so unmistakable in the period of diakinesis (fig. 56).

At a somewhat later stage the nuclei often possess one or more threads which show a partial or complete central longitudinal cleft. When this is present in Benacus it usually extends lengthwise completely through the thread. In Belostoma and Lethocerus, on the other hand, the diplotene threads are cleft only in certain regions (figs. 56, 58, 59). This condition has been more recently studied in the case of Lethocerus, and consequently its description will be drawn from this species. A typical diplotene nucleus (fig. 56) shows three relatively long, often partially split, threads, together with two which are much shorter and apparently never split. The two latter elements are the sex chromosomes, while the three larger ones correspond to the three pairs of spermatogonial autosomes. Another species of Lethocerus possesses a very marked cleft in the autosomal threads at this stage, and a drawing of one is introduced here for comparison (fig. 57).

The X- and Y-chromosomes in Benacus retain their compact form without significant change throughout the postsynaptic spireme stage. In this animal they constitute the most conspicuous elements in the nucleus. This is also true to a lesser extent in Belostoma, while in Lethocerus the sex chromosomes continue in a thread-like form for some time.

The similarities between Benacus and Pyrrhocoris (Gross, '06) are most striking in the diffuse stage and in those stages immediately following. Because of their peculiar nature, the changes undergone by the chromatin during this period will be somewhat more carefully described than in the case of the other forms. The approaching confused period is first indicated in Benacus when the diplotene threads begin to develop side branches and break up into granules (fig. 46). The threads gradually go over into a condition resembling the reticulum of resting cells (fig. 47). This is followed by a stage in which the chromatin is distributed throughout the nucleus in the form of much-attenuated threads (fig. 48). Following this is a long period in which the chromatin is gathered into a net characterized by threads often more or less parallel (fig. 49).

The next stage is very unexpected. Among investigators who have reported second contraction stages are Wilson in Largus ('12) and Gross in Pyrrhocoris ('06), both of whom placed it at the close of the diffuse period. Gross regarded this as the period of synapsis. Yocom ('23) found a similar stage in Leptocoris and concluded that it corresponds to the true synizesis of other forms, but that real synapsis occurs later. It would appear that Yocom's figure 3 represents the true synizesis, and the fact that he finds some evidence of duality in the threads at this time would be evidence that a real pairing has taken place regardless of what occurs later. After a very careful study of the matter, I have concluded that in Benacus the second contraction period is interpolated into the very middle of the diffuse period, and not at the end of it, where it is usually placed. In this respect my conclusions are in agreement with those of Yocom, whose figures

show the most diffuse period to follow, and not precede this stage. In contrast to the first contraction stage, the second shows a lack of definite threads and a highly granular condition of the chromatin together with definite centers of condensation in the contracted mass, and these exist in approximately the diploid number (figs. 50, 51). At the end of the period of contraction, which is not of long duration, the granules again move outward into the nuclear cavity and completely fill it with a lightly staining reticulum as in an earlier stage (figs. 48, 49). After a long resting period, during which little change occurs in the nucleus, the chromatin begins to aggregate into large irregular patches connected by linin threads (fig. 55). This marks the close of the diffuse period and the beginning of true prophase.

Synizesis stages often have been ascribed to poor fixation, and therefore are regarded as artifacts by some, and doubtless there will be those who will regard the successive contraction stages of Benacus in the same light. In regard to this question the following facts should be noted: the contraction stages appear following all fixatives; cells containing chromatin in the contracted state do not otherwise show evidence of poor fixation (poor fixation often shows on cytoplasmic inclusions first of all); when tissues of Benacus and Lethocerus are mounted together and given identical treatment at every step, the characteristic appearance of the chromatin occurs in every case.

The way in which the double threads of the diplotene stage are converted into the diffuse network is better shown in Lethocerus than in the other two species and will be briefly discussed here. The first step in this process is seen when the parallel threads separate by a lateral movement and begin a fine branching (figs. 58, 59). The separation does not involve the double thread along its total length for in places the two moieties of which it is composed remain attached as in figure 59. A coarse reticulum is formed by a continuation of this process (fig. 60).

No second contraction period occurs in either Belostoma or Lethocerus, and this again emphasizes the fact that closely related species may differ greatly in the details of maturation. In both of these animals the diffuse stage continues unbroken to the beginning of diakinesis.

Certain interesting differences may be noted in these species during the diffuse period in regard to the behavior of the sex chromosomes. In Benacus they continue as compact and deeply staining bodies throughout this period and can be identified even in the second contraction stage. Slight differences of form and size can sometimes be made out, but usually they appear equal in size in this animal. In Lethocerus the sex chromosomes first become definite compact bodies at about the time of the establishment of the diffuse period. From this time throughout the remainder of the maturation process they are never lost to view. The greatest difficulty has been encountered in tracing these chromosomes through this period in Belostoma. The Y- is much smaller than the X- and apparently is often covered up by its mate or by the deeply staining plasmosome. In all three species the sex chromosomes occur together more often than separate from each other, indicating a probable attraction for each other, although this is not sufficient to cause a true pairing during the synaptic period.

c. Diakinesis and the meiotic divisions. In these forms the first indication of the beginning of diakinesis appears when the chromatin gathers from its very diffuse condition into flaky masses lying close to the nuclear membrane (fig. 65). In Benacus and Belostoma these masses are always more compact than they are in Lethocerus, where they are particularly nebulous. Omitting the sex chromosomes from consideration, the haploid number of these loose chromatin masses is clearly demonstrable in Lethocerus and reasonably so in the other two species.

In Benacus the irregular masses of chromatin increase their staining capacity and rapidly condense into figures more or less cuboidal, and many of these acquire an opening

in the center (figs. 66, 68). At first separate centers of condensation, varying in number, arise; these are either reduced to an irregular ring or to a quadruple body by progressive fusion (figs. 68, 69). While the general behavior of the 'tetrads' during these changes is similar to that in Leptocoris (Yocom, '23), there is one important point of difference. Yocom has found the diploid number of chromatin masses at this time, while I find the haploid number. When the masses are viewed from a lateral aspect, a single mass may appear as two, more or less closely connected, for the same reason that an ordinary tetrad in a state of partial condensation may appear as two bodies, when in reality it is to be regarded as a single body of double origin. It is doubtful whether the kind of association in pairs following the diffuse period in Leptocoris can properly be called telosynapsis. The continued condensation results in the formation of thirteen compact bodies usually rather closely apposed to the nuclear membrane. Before these have time to complete their condensation, another contraction phase occurs, and all are crowded into the center of the nucleus (figs. 66, 71). Gross ('06) has interpreted this stage as one of synapsis in Pyrrhocoris and found the actual numerical reduction to take place at the same time. This cannot be the interpretation in Benacus. It is only a completion of the condensation of tetrads actually formed very much earlier. While thus crowded together, one of the tetrads very frequently occupies a position to one side and somewhat isolated from the rest (fig. 71). This is not the pair of sex chromosomes as might be expected, because these can frequently be seen within the mass in the center of the nucleus, still somewhat more deeply stained than the autosomes. After some time, each of these chromatin masses becomes dumb-bell-shaped, and at about this time the whole mass of chromosomes shifts its position from a central to an excentric position (fig. 72). Soon after this, the chromosomes move back into a peripheral position, the nuclear membrane breaks down (fig. 73), and the chromosomes place themselves on the developing spindle preparatory to reduc-

tion. As in other earlier stages in Benacus, the sex chromosomes are commonly found close together in the stage of diakinesis and are of more compact form and stain more deeply than the autosomes until after condensation has reduced the latter to their final form. At this time the sex chromosomes become indistinguishable and are not again identifiable until the metaphase of the second division, when their behavior discloses their identity.

The behavior of the chromatin during this stage in Lethocerus differs as markedly from that in Benacus as would be expected from an animal widely separated in taxonomic position. The nebulous masses produced from the diffuse network at the beginning of diakinesis soon assume the shape of large rings, always three in number. One of the rings appears to lead the other two in condensing and is also somewhat the largest, its diameter being about two-thirds that of the nucleus (fig. 75). As condensation proceeds, it becomes clear that each ring is divided into two by a split running parallel to the plane of the ring (fig. 76). This double ring is also divided transversely into two double rings (fig. 78). At times the points of union of the half-rings show prominent 'lugs' such as are frequently described by students of spermatogenesis (fig. 78). Condensation is continued until each double ring is reduced to such a structure as is shown in figure 78, f, which closely resembles two spermatogonial chromosomes with their concave surfaces apposed. These bodies rapidly condense into dumb-bell figures (fig. 80) and ordinarily go onto the spindle in that condition, but in some cases the rings fail to close up entirely before taking their places in the equatorial plates (fig. 83). In these cases the behavior of the rings is closely similar to the behavior of the ring tetrads in Cicada (Shaffer, '20). A study of figures 78, 80, etc., will make it clear that these ring tetrads are all atelomitic of the sort described by Carothers ('17) in Trimerotropis and Circotettix. Although ordinarily the rings condense into chromosomes without twisting (fig. 78), occasionally one may be found which is apparently twisted into a figure 8 (fig. 77).

A careful examination in these cases shows that the figure 8 is caused by a distortion of the ring looked at from a lateral aspect. There is clearly no 'chiasma' at the point of apparent crossing, indeed, the threads do not even touch at this point. Such twisted rings straighten out in the process of condensation as in other cases. No evidence exists of a massing of the chromosomes just previous to the breaking down of the nuclear membrane as in Benacus, and the completed chromosomes retain their perimembranal position until the spindle is well along in development. The sex chromosomes are never lost to view throughout diakinesis. During the latter part of the period they always occur separate from each other and at this time undergo a constriction, doubtless representing a longitudinal division, and rapidly become converted into the usual dumb-bell-shaped bodies. In no case have the sex chromosomes been found to show a second split at any stage of their development (figs. 80, 81, 83).

As shown in a former paper ('16) the tetrads in Belostoma are more varied in type than in Lethocerus and Benacus. Two large rings are constantly present, and these are to be regarded as corresponding to the four large chromosomes in spermatogonia. V's and rods, often twisted, also occur. In all probability these are all to be traced back to the double threads of the diplotene stage. All tetrads here are finally reduced to dumb-bell figures as in the other two species (fig. 87). Just previous to the formation of the first spindle and the dissolution of the nuclear membrane, the chromosomes crowd together into a compact group at the side of the nucleus and there form a conspicuous feature as in Benacus (fig. 84).

During the maturation divisions in these animals there is very little tendency for the chromosomes to become obscured by clumping together except, possibly, during interkinesis in Benacus and Belostoma. This fact makes these animals favorable objects for the study of chromosome behavior during this stage.

In the metaphase of the first division it becomes certain that there is always one more than the haploid number of

chromosomes present in all three species. This is due to the presence of the XY-pair of sex chromosomes.

Polar views of the primary spermatocytes of Benacus usually show thirteen prominent chromosomes, varying in size, together with two which are considerably smaller; one of these smaller chromosomes is very small and usually lies at some distance from the main group (figs. 89, 90, 92). Deviation from the normal number is usually caused by a loss of the smallest chromosome, although rarely one or more of the others may be missing. Plus deviations occur only very rarely, and in most cases these seem to be caused by the presence of chromatoid bodies which have taken up a position close to, or even among, the chromosomes. In general, the arrangement of the chromosomes is irregular in the equatorial plate, but at times there is a tendency to form a ring with three or four within the circle (fig. 91). Attempts to identify the sex chromosomes in this stage have been fruitless, but they must be a pair of intermediate size.

In the case of Belostoma there is little to add to my original account of the chromosomes at this stage. The number is very clearly thirteen (figs. 96, 97). Among these are two which are much larger than the remaining ones and doubtless correspond to the four large spermatogonial chromosomes. The arrangement of the chromosomes in the equatorial plate is irregular as in Benacus.

In Lethocerus five chromosomes appear in all normal metaphases of the first division and can be identified at every step both in lateral and polar views. Three are clearly bivalent, while the two small chromosomes are just as clearly univalent. The bivalent chromosomes often continue to show the double condition after being placed on the spindle. The small sex chromosomes always occur close together in the plate and thus resemble those of Oncopeltus and Lygaeus (Wilson, '12).

A study of lateral views of metaphase show that all chromosomes divide in the first division, with the result that all secondary spermatocytes receive the same number as the primary spermatocytes possess (figs. 92, 93, 99, 100, 113).

Polar views of anaphase stages show that each secondary spermatocyte has fifteen chromosomes in Benacus, thirteen in Belostoma, and five in Lethocerus (figs. 95, 101, 113). No significant changes occur in the relative position of the different chromosomes during their passage to the poles of the spindle in this division. In certain cells at this stage in Benacus and Belostoma two chromosomes often divide a little before the rest and these may be the sex chromosomes. In Lethocerus the two sex chromosomes always precede the autosomes in division (figs. 111, 112).

It should be clear from the description of tetrad formation in Lethocerus that the first division is reductional for the autosomes and equational for the sex chromosomes. This is practically certain for Lethocerus and also the most probable in the other two forms, although the matter will remain obscure in Benacus, due to the very peculiar mode of tetrad formation.

During the period of interkinesis, the conjugation of the sex chromosomes occurs in all three species, together with a more or less marked rearrangement of the autosomes. In Benacus the larger number of chromosomes and their crowded condition in this stage makes it difficult to observe the phenomenon directly in this species (figs. 102, 104), but there can be no question of its occurrence. In Belostoma the slightly smaller number of chromosomes and their size relationships make it somewhat less difficult to follow the changes undergone by the XY-pair in this stage. Extended work on this species has made it possible to follow every step in the conjugation process which does not differ essentially from that in Lethocerus. In the latter animal it is perfectly clear that no essential change occurs in the grouping of the chromosomes until late anaphase (fig. 112). From this time on the two sex chromosomes slowly approach each other and finally come into contact (figs. 113, 114, 115). This contact is maintained until the final disjunction of these elements in the following metaphase.

The duality of each diad becomes clearly delineated during interkinesis in Lethocerus and is sometimes indicated in the other two species. As the single chromosomes separate in the first division anaphase, the cleft dividing the halves of the chromosomes from each other becomes much more marked. At this time the diads have the appearance of pairs of chromosomes lying side by side (figs. 113, 115). The marked cleft becomes the constriction which separates the single element into two in the following metaphase (figs. 117, 118).

In all cases when the chromosomes become aligned in the equatorial plate preparatory to the second division exactly the haploid number is visible in polar views, fourteen in Benacus, twelve in Belostoma, and four in Lethocerus (figs. 105, 128, 111). Lateral views of the second metaphase show the XY-pair arranged end to end (figs. 107, 129, 118).

In the second division the size relationships of the sex chromosomes come out most clearly. The X- and Y-chromosomes in Benacus are usually so nearly the same in respect to size as to be indistinguishable from each other. Only occasionally is one somewhat larger than the other (fig. 107). In Belostoma there is always a marked difference in respect to size (fig. 129). By analogy with other forms, in which the chromosomes are known for the female, the smaller may be called the Y-chromosome. In this species the secondary spermatocytes always have their chromosomes arranged in a ring with eleven autosomes surrounding the pair of sex chromosomes occupying the center. This new arrangement is accomplished during the period of interkinesis by an obscure mechanism so far not analyzed. In Lethocerus the sex chromosomes are always of almost exactly the same size.

The sex chromosomes almost invariably disjoin in the second division and start on their journey toward the spindle poles before the autosomes are divided (figs. 107, 129, 118). Especially in Lethocerus, but also in the others, the sex chromosomes maintain their advanced position and reach the poles somewhat in advance of the autosomes (fig. 119).

A study of polar views of dividing secondary spermatocytes during anaphase stages shows that in every case the spermatids receive exactly half the spermatogonial number of chromosomes. In Benacus each spermatid gets fourteen chromosomes, including one of the sex chromosomes, but the two types of cells thus established are indistinguishable from each other. In Belostoma each spermatid receives twelve chromosomes, of which one is either the X- or the Y-chromosome (figs. 131, 132). The two types of cells thus established are readily identifiable. Hence, among these species Belostoma stands in the same class as Nezara and Lygaeus (Wilson, '05, '06, '11, '12), while Benacus and Lethocerus are to be classed with Oncopeltus in which there is no dimorphism of spermatozoa due to a difference in size of sex chromosomes.

d. Spermatids. The behavior of the chromosomes in the spermatids is quite regular and without special features in all three species here dealt with. Except in Lethocerus, the outline of individual chromosomes is lost in the telophase stage of the second division, due to crowding (figs. 137, 142). In this animal, however, the four chromosomes of each spermatid can be followed accurately for a long time (fig. 143). The autosomes are the first to lose their definite boundaries as the characteristic reticulum of the new nucleus is built. The sex chromosomes retain their more compact form and definite outlines for a much longer time (fig. 145). Near the close of this period and soon after the young spermatozoa are formed, the chromatin begins to take up a position just inside of, and closely applied to, the nuclear membrane, where it remains and later forms a distinct shell surrounding the nuclear cavity (fig. 153).

II. Cytoplasmic inclusions

a. Chondriosomes. 1. Spermatogonia. In all three species the chondriosomes have been traced throughout the spermatogonial divisions. They are always granular, only becoming filamentary after the cells enter the maturation period.

Bowen ('20) believed the chondriosomes to be both granules and threads in the Pentatomidae, while Montgomery ('11) was doubtful of their existence at all. In these animals they seldom stain brilliantly in this stage, but nevertheless in good Benda preparations they become clearly visible (figs. 6, 11 to 18).

In resting cells the chondriosomes are usually massed together, often at the distal pole of the nucleus close to its membrane (figs. 12 to 16, etc). During prophase stages the chondriosomes migrate throughout the cytoplasm (figs. 20, 21). Although very small and difficult to study, at times they appear to show a vesicular structure somewhat as shown by Gatenby in Palamnaeus ('25) and in several Lepidoptera ('17) and Pulmonata ('18).

As the mitotic spindle develops in preparation for division, the chondriosomes become arranged in a granular sheath or mantle almost entirely surrounding this structure (fig. 8). Small areas are always left free of granules at the spindle poles. In the anaphase stage the chondriosomes flow inward between the diverging groups of chromosomes and tend to crowd about the developing mitosome (fig. 18). Not all of these bodies behave in this manner, since many are left scattered about in the cytoplasm in random positions. Later, all gather into one or more masses and again become closely applied to the nuclear membrane (figs. 14, 15, etc.). It seems probable that chondriokinesis occurs at some stage of this process, because there is no apparent diminution in the number of chondriosomes per cell during successive mitoses. Small granules may repeatedly be found in positions suggesting division, but a settlement of this question must await further study.

The large tetraploid cells found so frequently in the walls of follicles possess the same granular type of chondriosomes as do the spermatogonial cells. They are here larger, more numerous, and often form a dense perinuclear zone in the resting stage (fig. 25).

2. The growth period. In the fully established daughter cell following the last spermatogonial division the chondriosomes occupy their usual position in one or more cap-like masses close to the nucleus. As the leptotene stage is approached the cap-like mass loosens up and assumes a more spherical shape (fig. 33). These changes cause a rather marked transition in the follicle between the region occupied by spermatogonia and that filled by cells in the leptotene stage. The loose ball of chondriosomes becomes more definitely organized as development proceeds until, by the time of synizesis, a conspicuous structure occupies the cytoplasmic pole of the cell (figs. 30, 39, 40). The center of the ball shows some evidence that threads are being formed within it, but these do not become clearly evident until considerably later. There is little change in the state of chondriosomes throughout the pachytene and diplotene stages (figs. 44, 54), but as the diffuse stage approaches they tend to move into the surrounding cytoplasm. As the chondriosomes become more freely dispersed in this manner, it is clearly seen that they are now thin and delicate threads (figs. 52, 54, 55). The method by which the threads are formed is obscure, but there is little doubt that they are formed by a fusion of the granules. Several recent investigators, working on fixed material, have reported the development of threads from granules by some kind of fusion process. Among these is Gatenby ('17), who has reported this phenomenon among Lepidoptera. He thought it possible to hasten the fusion by use of certain fixatives. Shaffer ('20) found that granular mitochondria in the spermatogonia of Cicada were replaced by filar mitochondria in the pachytene stage. Bowen ('20) found the granular chondriosomes in the Pentatomidae becoming thread-like in later stages, but was uncertain as to the exact method by which the change was accomplished. Hyman ('23) has definitely determined that the thread-like mitochondria in the male cells of Fasciolaria tulipa arise by a fusion of granules. Voinov ('16, '25) has very clearly described the fusion of granules to form a long filamentary chondriosome in Gryllo-

talpa vulgaris. The facts in the Belostomatidae appear to be in agreement with these cases cited above.

3. Diakinesis and the meiotic divisions. The chondriosomes attain their greatest prominence during this stage, due to the completion of the fusion of granules into threads as heretofore described. Quite marked differences in regard to the extent of formation of threads, staining reactions of the chondriosomes, position assumed in the cell, and extent of granulation of the chondriosomal threads have been noted among these animals.

Chondriosomes reach their greatest prominence in Benacus. By the time the incipient tetrads are well along in development, the granular chondriosomes have all given rise to the filamentary type, almost completely enveloping the nucleus with a dense hedge of deeply staining, apparently homogeneous threads (fig. 70). Whether these threads migrate or are moved outward by cytoplasmic currents is an interesting question not readily answered by ordinary cytological methods. At any rate, a movement of the threads takes place with the result that the cytoplasm becomes generally filled with them. Chondriosomes in the living cell appear to be in constant motion, according to the Lewises ('15). If this is true in these cells, it must be that the range of their movement is much increased during this stage, because formerly they are always found restricted to one pole of the cell.

In both Lethocerus and Belostoma there is the same fusion of granules to form threads as in Benacus. There is, however, a later tendency for them to fragment again, so that by the time the spindle is formed there are both granules and threads present (figs. 79, 84). Fragmentation is accompanied by a reduction in staining capacity of the chondriosomes, and this prevents these bodies from coming out as plainly in the maturation divisions as in Benacus. Lewis and Robertson ('16) have seen chondriosomes fragmenting in living cells of Chorthippus. In certain cysts the chondriosomes retain their thread-like form and closely resemble those of Benacus.

The chondriosomes in Benacus retain their thread-like form essentially unchanged throughout the maturation divisions and continue to stain with striking brilliance following the proper treatment. In the prophase of the first division they become placed in the form of a sheath surrounding the developing spindle and nearly filling the cytoplasm of this region. In metaphase the chondriosomal threads appear to be somewhat oriented around the centrioles (figs. 91, 93), but this is very obscure, due to the interlacing of the threads. In the division of the primary spermatocyte (fig. 103) the chondriosomes appear to be sorted out into two approximately equal masses and thus delivered to the secondary spermatocytes. Sometimes individual threads appear to be caught between the mitosome and the cell membrane and cut in two. During interkinesis they retain their individuality completely and show no tendency to agglutinate or to fragment. In the second division they behave essentially as in the first and are again sorted out into two equal groups for the spermatids. This time they are more likely to be caught midway between the two cells and severed much as described by Bowen ('20, '22).

Because of fragmentation and loss of staining power formerly noted in Belostoma and Lethocerus, it is much more difficult to follow the chondriosomes through the maturation divisions in these animals than in Benacus where the conditions are so clear and unmistakable. In spite of this obscurity in their behavior, careful observations clearly indicate that the steps in these divisions are essentially identical in all three forms and that in all cases the original complement of chondriosomal material in the primary spermatocyte is sorted out into equal masses in the spermatids as in Benacus.

4. Spermatids. As the telophase of the second division advances, the chondriosomes collect in a mass of threads located between the group of chromosomes on the one hand and the midbody on the other. They soon form a ring about the mitosome (figs. 138, 139, 140). The ring-like mass of threads gradually condenses into a spherical body of rather

loose texture—the nebenkern. After the ring of chondriosomes has been molded about the mitosome it rotates outward to free itself from this structure, according to Bowen's ('22) observations. In this way the nebenkern comes to lie on the side of the nucleus opposite to that occupied by the original sheath of threads in the Pentatomidae. This does not appear to be true of the Belostomatidae. In the members of this family so far studied the condensation of the nebenkern appears to take place around the distal end of the mitosome and becomes free from this structure when the latter disintegrates. Thus the nebenkern retains its original position between the developing nucleus and midbody. During the condensation of the nebenkern many of the curious patterns so frequently observed and more or less carefully reported by numerous authors have been observed in the spermatids of these animals. While the description of the formation of the nebenkern given above is based primarily upon observations in Benacus, the facts are essentially the same in the other two species. In those cases where the chondriosomes come through the maturation divisions unfragmented their behavior in Belostoma and Lethocerus is practically identical with that in Benacus. In cases where their behavior is more or less obscured in these species the end result appears to be the same (figs. 144 to 146).

The nebenkern remains in a spherical form without clear differentiation into two substances for a considerable time. In material stained according to Benda very little can be seen of internal organization, but in material fixed in Flemming's fluid and stained with iron-hematoxylin it is clear that the interior of this body is vacuolated during a part of this period (fig. 147). At about the time of the reappearance of the centrioles the surface of the nebenkern can be seen to be separated somewhat from the central portion (fig. 149). This is the beginning of a marked differentiation into an outer chromophobic and an inner chromophilic portion characterizing the later steps in this stage (figs. 151, 152). The central deeply staining portion continues to condense until there is

a broad zone separating the chromophilic from the chromophobic part (fig. 153). Often a second still more deeply staining central region of the chromophilic portion can be seen at this time, indicating internal changes within this part. This is the condition of the nebenkern at the time it is ready to divide in preparation for elongation into a sheath for the tail filament.

b. Golgi bodies. 1. Spermatogonia. The Golgi apparatus exists in spermatogonia as a series of separate, minute granules probably plate-like in form. Their continuity from cell generation to cell generation appears to be a certainty. When first seen they are scattered about the nuclear membrane usually closely applied to its surface (figs. 12 to 14, 17, 19), but may be crowded into a group among the chondriosomes (figs. 12, 17, 22). Accurate determination of their structure is difficult at this stage. Superior modified Kopsch preparations (fig. 19) show a form similar to that shown so clearly during the middle growth period. Thus it is probable that these bodies have the same fundamental structure in spermatogonia as they do at the time of their greatest size.

During mitosis the Golgi bodies break up into numerous smaller pieces dispersed freely among the chondriosomes. In Mann-Kopsch preparations the minute Golgi pieces come out as intensely black granules just as they do in the later maturation divisions, except that they are smaller and more difficult to identify. They appear to fuse again in telophase stages to form the Golgi bodies of the resting cell.

The number of separate Golgi bodies present in each cell cannot be definitely stated, but is probably about twenty. No fusion of these into a compact body, such as described by Bowen ('20), has been observed. The body regarded by Montgomery as an idiosome is a cap of chondriosomes partly destroyed by the fixative, as pointed out by Bowen. The same can be stated of Gross's work on Pyrrhocoris ('06) and that of Paulmier on Anasa ('99), where the mitosome was mistaken for the idiosome. Bowen ('20, '22) was the first to clear up the confusion surrounding these structures in the Hemiptera, and the present work confirms his view.

Prominent Golgi bodies occur in the large tetraploid somatic cells (figs. 23, 24). In Benacus they are particularly large; here even following fixation in Flemming's strong fluid they show their characteristic form and structure very clearly.

2. Growth period. The fragments of Golgi bodies delivered over to the primary spermatocytes in the last spermatogonial division apparently fuse into a few larger elements as in former divisions. Growth begins in these early in the maturation process, so that by the time of synizesis their typical form and structure can be clearly seen (figs. 30, 33, 40, 44, 46). Belostoma has been the most useful in studies on the Golgi bodies, but observations on this form have been fully confirmed by work on the other two species. Growth continues until about the middle of the diffuse period when these elements reach their maximum size when they appear most favorable for a study of their form.

As Bowen ('20) has pointed out, Golgi bodies are not normally vesicles, but platelets, more or less horseshoe-like in shape, although this form may be considerably modified in certain individuals. In good preparations a central lightly staining substance can be seen enclosed by the more deeply staining rim (fig. 64). About as often as would be expected on a basis of chance, single straight rods appear among the horseshoe-like platelets. These are probably the same bodies seen from the lateral aspect. At times two rods appear nearly parallel or diverging at an acute angle. These are to be regarded as rather extreme variations of the horseshoe shape and not as an indication of a divided condition as seen from the edge as held by Bowen ('20). Golgi bodies prepared according to the Mann-Kopsch technique usually tend to be overstained or irregularly stained. This is illustrated in figure 61 taken from Lethocerus. Two of the Golgi bodies bear smaller granules upon their outer surface. At times these seem to be replicas of the larger bodies and at other times they appear like branches or canals from them. Their significance is not clear.

Of more than passing interest are the Golgi bodies found in one individual Belostoma. In all stages of maturation they were considerably enlarged and vesicular in form instead of plate-like. Although the testes of this animal were fixed in Flemming's strong fluid which would ordinarily render them inconspicuous, the Golgi elements stood out even more clearly than in special preparations, indicating a change in their chemical make-up. A small percentage of these bodies were normal in shape, but most of them were vesicular and many were in the form of double-walled cups (fig. 63). The chromosomes in this individual appeared entirely normal, but the chondriosomes showed the same resistance to the action of acetic as noted for the Golgi bodies.

3. Diakinesis and the meiotic divisions. The period of diakinesis is also a critical period in the history of the Golgi bodies. For reasons already stated, material for the study of this important stage has been confined to Belostoma and Lethocerus, but there is no reason to believe that the facts are different in Benacus. As is well known to cytologists using the Mann-Kopsch technique, it is often difficult to establish the exact seriation of stages under observation, because the chromatin, which furnishes the best guide, fades out more or less completely. The facts relating to the Golgi bodies during the period of diakinesis appear to be as follows: these bodies maintain their definite form and plate-like structure until about the time the first spindle begins to form, when they fragment into a large number of very minute dictyosomes (fig. 86). Then the dictyosomes spread generally throughout the cytoplasm (fig. 88).

In the metaphase of the first division the dictyosomes, produced by fragmentation of the Golgi bodies, are massed in a zone just outside of the equatorial plate of chromosomes and more or less intermixed with chondriosomes (fig. 98). In certain preparations both of these are visible at the same time, but in order to differentiate clearly special methods must be used. As division occurs the dictyosomes are sorted out into two approximately equal groups (fig. 127). No evi-

dence exists of further division of the dictyosomes after the rapid mass-fragmentation formerly described. Occasionally some of the largest elements show a vesicular or ring-like form, but in general they are rod-like or plate-like, the finer structural details of which are invisible. During interkinesis the dictyosomes apparently lie unchanged in the cytoplasm, but they soon come under the influence of the centrioles again and are arranged around the second spindle as in the first division. During the anaphase of this division they are again sorted out into the daughter cells essentially as before (fig. 134). As noted heretofore, the most careful work on Golgi bodies has been carried out in Belostoma and Lethocerus, but there is every reason to believe that their behavior is fundamentally the same in Benacus.

4. Spermatids. The dictyosomes, delivered to the spermatids in the second maturation division, remain irregularly distributed in the cytoplasm for some time during the early stages in the reorganization of the nucleus and the condensation of the nebenkern. Probably some fusion of the smaller dictyosomes occurs giving rise to larger units. At first the dictyosomes are only visible in specially prepared material, but later, after fusion has progressed somewhat further (fig. 148), they can be clearly seen in most preparations. Eventually the dictyosomes fuse to form a single body—the acroblast. For a time just before the formation of the acroblast the dictyosomes are usually grouped in the close vicinity of the nebenkern, but they do not seem to be actually fastened to its surface as in Brochymena, but more nearly resemble Murgantia and Euschistus (Bowen, '22) in this respect.

In the fully established acroblast two portions can be clearly seen—a central lightly staining mass surrounded by a rim of deeply staining material. In most cells the acroblast is more vesicular than were the separate Golgi bodies of the primary spermatocyte. At first this body appears not to have a definite position in the cell (figs. 147 to 152), but as this stage draws to a close it comes to lie in the angle between the nucleus and the nebenkern opposite the side occupied by the

centrioles. Bowen ('22) has shown that the acroblast, after lying for a short time in the position just mentioned, migrates to an anterior position usually simultaneously with the posterior migration of the centrioles. In the Belostomatidae there is also a migration to an anterior position at or near the close of this stage, but this migration appears to be less regular and precise than in the Pentatomidae. Figure 153 represents two young sperm of Belostoma at the close of this stage. In one cell the acroblast has already migrated to the anterior end of the sperm, while in the other this body is still in its temporary resting place at the base of the nucleus. In many young sperm of this stage the acroblast shows a minute and deeply staining granule within the light area and usually attached to the rim of deeply staining material. This is doubtless closely connected to the formation of the acrosome. It is hoped to trace its further history in a later paper.

c. Minor cytoplasmic inclusions. 1. Centrioles. The centrosome is represented in the resting spermatogonia by a single minute centriole, with no visible differentiated cytoplasm around it. Its minute size and the presence of numerous cytoplasmic granules cause considerable difficulty in tracing it throughout the whole mitosis. From the time of its division in middle prophase the centriole can be clearly traced until it again nearly fades from view in late anaphase or early telophase. In certain preparations a minute granule can often be found hugging the nuclear membrane, but it is difficult to differentiate it from the chromatoid bodies occurring in its vicinity. The height of its staining power is reached during late prophase during the formation of the spindle.

At the outset of the process of maturation the centriole rapidly loses its staining power and fades into obscurity not to emerge into prominence again until diakinesis. Following Bouin's fluid or other similar reagents, the centriole can occasionally be seen during the long diffuse period, but its positive identification is uncertain.

During the latter part of diakinesis the centrioles again come into clear view in all three species (fig. 67). They are

visible for some time as minute dots before astral rays are visible around them. At first they take the stain but slightly and appear much smaller than at a later period, but this may be because of their weak staining power. At this time they are already on opposite sides of the nucleus, though not 180° apart.

As usual, the centrioles are divided during the prophase of the first division in preparation for the second (figs. 80, 83, 87, 92, etc.). In late anaphase of the first division the two centrioles in each secondary spermatocyte decrease in staining power for a time, while they separate and take up their new positions for the second division (figs. 100, 102, 104, 112, 114). It is still impossible to state how the centrioles influence the orientation of the group of chromosomes so that the position of the latter is at right angles to the former position, but it seems probable that the new arrangement is brought about by individual movement of the chromosomes under the influence of the centrioles. During the later prophase of the second division the centrioles again stain more prominently and sometimes show signs of duality, although this is doubtful. Following the second division, these elements lose their staining power or markedly diminish in size and gradually fade from view only to become visible again during the early stages of sperm formation.

At about the time the nebenkern shows its first signs of differentiation into two distinct portions the centrioles come into clear view again. They may appear as a pair of granules or as a rod and, in general, closely resemble the centrioles described by Bowen ('20, '22) in the Pentatomidae. Their position when first seen is always at least 90° from the developing nebenkern and may be practically at the extreme anterior end of the cell (figs. 147, 149, 150). No tail filament is at first distinguishable in connection with the centrioles, but shortly after this one is invariably present (fig. 150). Soon after the appearance of the short tail filament the centrioles and the attached filament migrate posteriorly and medially to their permanent resting place at the base of the

nucleus (figs. 151, 152). In his very painstaking study of these stages in the Pentatomidae Bowen ('22) has concluded that the centrioles change their relative position just before migration, so that both lie against the nuclear membrane. They then migrate across the face of the nucleus in a path coinciding with the plane of subsequent division of the nebenkern. This allows the tail filament easily to find its place between the halves of the nebenkern. While the Belostomatidae have not as yet been studied as carefully as the Pentatomidae, the facts at present strongly confirm the work of Bowen in most points.

2. Mitosomes. The mitosome in Hemiptera is usually regarded as a very transitory structure, passing out of existence soon after each division. It has frequently been noted as a more or less prominent element, depending upon the technique used, however, as with Henking ('91) in Pyrrhocoris, Paulmier ('99) in Anasa, and Montgomery ('11) in Euschistus, and sometimes mistaken for other structures as in the case of Gross ('06), who regarded it as an idiosome in Pyrrhocoris. In Benda preparations whole cysts are often found in the Belostomatidae, in which all the cells are bound together by mitosomes and midbodies in a most intimate manner (figs. 12 to 15). In late anaphase the fibers left from the spindle are few, but these rapidly increase in number and darkly staining granules are laid down at the junction of the fibers and the new cell membrane formed between the daughter cells (fig. 18). The granules mark the beginning of the midbody (fig. 14). Figure 15 represents eight cells belonging to a cyst of about thirty-two cells, all of which were bound together by this intricate system of bridges in much the same manner as described in the spermatogonia of Salamandra maculosa (Meves, '99), Leptinotarsa (Hegner, '14), Vespa (Maziarski, '13), and others. For some time after the mitosome is formed it is free of granules and fibers within. At this time certain sections show it as a prominent ring with mitochondria crowded around its periphery and accentuating its ring-like appearance in cross-section (fig. 13).

The prominent mitosomes of the spermatogonial period seem to disappear shortly after the onset of the leptotene stage. In fact, the mitosome of the last division is probably never completely developed and disappears soon after formation. Remains of midbodies may be found attached to the periphery of cells at the leptotene stage, but careful search fails to disclose the mitosome itself. Cells formerly bound together by these structures are now irregularly placed with respect to each other.

Following the first maturation division, a conspicuous, barrel-shaped bundle of fibers is developed representing the mitosome (figs. 102, 112). This structure rapidly lengthens and soon becomes constricted (figs. 104, 114, 126). A few granules appear at the place of constriction, but these never develop into a prominent midbody at this stage and soon disappear from the cell along with the mitosome.

During the telophase of the second division a very conspicuous mitosome is built up, together with a well-defined midbody. These structures persist for a relatively long time during the changes in the transformation of the spermatid into young sperm. During the later telophase stages a marked elongation of the mitosome occurs (figs. 135, 142, 144, 145). Curved mitosomes are commonly produced during the process of elongation (fig. 142) in Belostoma and Lethocerus, but are rare in Benacus. As suggested by Bowen ('22), one gets the impression from observing these structures that the nuclei are more or less pushed about and possibly rather definitely rotated by the elongating mitosomes. The regularity of movement as described by this investigator has not been found in these animals, however. In many cases in Belostoma and Lethocerus one end of the mitosome appears to slip by the corresponding chromosome group, while the other end remains attached to or braced against the opposite group of chromosomes (figs. 144, 145). The midbody is well developed in the older spermatids in all three species. Its typical shape is that of a ring through which a small bundle of fibers extend into the daughter cells. About the time the

spermatids break apart preparatory to their characteristic orientation around the walls of the tubules, the midbody is cast off. Following this, the mitosome is gradually removed from the cell and in somewhat later stages does not appear at all among the constituents of the young sperm.

3. Chromatoid bodies. Chromatoid bodies have been definitely located in Belostoma and Lethocerus and probably also in Benacus as early as the rosette stage of the spermatogonia, whereas formerly (Chickering, '16) they were not found earlier than the confused stage of the chromatin. During the spermatogonial mitoses they are drawn into the vicinity of the equatorial plate and pass into the daughter cells according to their chance position, although most cells appear to possess one or more (figs. 6, 7).

Throughout the growth period chromatoid bodies are present, but the only noticeable change in them is a considerable increase in size (figs. 37, 39, 40). This is especially marked in Belostoma where some of them are as large as the smaller sex chromosome (fig. 62).

During diakinesis these bodies may be present up to six or eight in number, and they often appear to be in a state of division (figs. 67, 72, 73, 74, 80). After the breakdown of the nuclear membrane, one or more of the chromatoid bodies may be found among the chromosomes, when they more or less closely simulate them (fig. 83).

Chromatoid bodies are present in many and possibly all spermatids, but their behavior continues irregular except in one particular. In Belostoma the centrioles are accompanied by another granule during their migration to the base of the sperm nucleus (figs. 152, 153). This granule is relatively large and often obscures the centrioles, making it difficult to follow them through this period of their history. It is probably to be regarded as a chromatoid body and appears to be passed out of the sperm cell along with other similar bodies at a later stage not treated in this paper.

DISCUSSION

I. Synapsis

The problem of synapsis in animals and plants continues to be one of the most important in the field of cytology, even after more than a quarter of a century of intensive work on the part of many investigators. Many of the most important lines of investigation upon which biologists are centering their attention are in some manner more or less closely bound up with the question of the union of homologous chromosomes during the early maturation phenomena.

Although the actual fact of reduction of the chromosomes was discovered by Van Beneden in 1883 in Ascaris and by Strassburger in 1888, it was not until the work of Rückert ('92, '94), Haecker ('92, '95), and Vom Rath ('92) that ideas relating to the particular mode of synapsis began to take shape. These men appear to have regarded reduction as due to an end-to-end association of all the chromosomes in a continuous spireme followed by a segmentation into the haploid number of chromosomes (Wilson, '25 a).

Beginning with Winiwarter ('00), more precise notions of the mode of reduction came into being. In 1900 and particularly later (Winiwarter and de Saintmont, '09), Winiwarter developed the theory of parasynapsis. Since that date many of the most able cytologists have found confirmatory evidence of Winiwarter's conclusion in a large number of animals and plants.

The opposed theory of telosynapsis was elaborated by Montgomery ('00–'05) and Farmer and Moore ('03, '05, and later). A number of cytologists have supported this theory as a general mode of synapsis, and at the present time in certain special cases telosynapsis seems to accord best with the observed facts.

The history of the parallel development of these two rather sharply opposed views shows clearly that the advocates of telosynapsis, as a general mode of conjugation of homologous chromosomes, have steadily lost ground during the last twenty

years. New cases of telosynapsis have been reported frequently, however.

This whole question of synapsis has been reviewed very completely by Gelei ('21, '22) and Bouin ('25), and hence it is not deemed necessary to enter into any lengthy discussion of the recent literature in this paper. Certain tendencies, however, may be pointed out again and the present status of the question stated.

Gelei ('21, '22) has discovered exceptionally favorable conditions for the observation and interpretation of synapsis in the triclad worm, Dendrocoelum lacteum. Gelei has been able to show in the clearest manner that, following the lost oogonial division, the leptotene nucleus contains the full diploid number of chromatic threads—fourteen in this case. These become oriented under the influence of the centrioles in the form of loops in a typical bouquet stage. Following this orientation, the threads pair side by side, and this consists essentially of a side-by-side union of homologous chromomeres, which are especially clear in the Dendrocoelum. There is always a clear line of demarcation between the conjugating threads in this animal, and this line of separation becomes the splitting plane of the double chromosomes at the first maturation division. It is very difficult to understand how this can be interpreted otherwise than as parasynapsis. Gelei thinks Dendrocoelum is even more favorable for a study of this kind than the classical Tomopteris (A. and K. Schreiner, '06), and this appears to be true, and thus furnishes an exceedingly strong confirmation of the work of the Schreiners.

Janssens' ('24) recent work on two species of Orthoptera also shows particularly clear cases of parasynapsis. In Stethophyma grossum the leptotene threads become gradually oriented toward the side of the nucleus near which the centriole occurs. There is a very clear differentiation in the lengths of the individual threads corresponding to the spermatogonial chromosomes. No loops are formed in this animal, but cross-sections of the bundle of leptotene threads

show the full diploid number. Soon the leptotene threads form 'twin associations,' and a little later each twin becomes secondarily split, forming quadruplets, which are, of course, the definitive tetrads. Evidence of parasynapsis has also come from Janssens' study of Chorthippus parallelus, which seems to resemble the batrachians in this respect which formed the object of his original investigations. The amphitene stages so clearly shown in this material can only be adequately interpreted as due to parasynapsis. This work added to his earlier work on the same subject makes a strong case for this type of conjugation.

The recent work of Bouin ('25) is of interest in connection with synapsis in several ways. The claims of Winiwarter and Gregoire relative to parasynapsis have induced Bouin to return to a study of the Myriapods for a reexamination and reinterpretation of his former observations. Although formerly ('02) this worker favored the telosynaptic view, this study of Scolopendra cingulata and the reexamination of the material of his former study has convinced him of the truth of parasynapsis. According to the present observations, there is a very pronounced growth period interpolated into the history of the primary spermatocytes and this greatly complicates the interpretation of the stages. Nevertheless, it seems clear that the gonial chromosomes are transformed into leptotene threads, and that these then become oriented about the centriole in a bouquet stage. Parasynapsis then occurs rapidly in the usual manner.

The occurrence of parasynapsis among the Hemiptera-Heteroptera seems to be clearly established as the ordinary mode of union of homologous chromosomes in this group. This group of animals has not been a favorable group for the study of this phenomenon, however, because of the pronounced synizesis and often a prolonged confused stage which is interpolated into the history of the chromatin. Although, as already pointed out, an earlier advocate of the telosynaptic theory, Montgomery reversed his findings in 1911 as a result of a very detailed study of the behavior of the chromosomes

in Euschistus. In this work he showed that what he had formerly regarded as a line of longitudinal splitting of single chromosomes is in reality the line of conjugation between two whole chromosomes, and thus his conclusion was that parasynapsis is the usual mode of synapsis.

Wilson ('12) made a very painstaking study of several Hemiptera in respect to the question of synapsis. In this work great difficulty was experienced in tracing the behavior of the chromatin because of the stage of synizesis and marked growth period. Wilson came to the conclusion that this question is practically insoluble in such animals. However, through a study of the process in Tomopteris and in Batrachoseps, which formed the basis for the ideas of the Schreiners ('06) and Janssens ('05) and even an examination of the original material used by these workers, Wilson became convinced that synapsis is a fact and that it is carried out (at least in these forms) by a side-by-side union. Furthermore, he believed that the facts observed in Oncopeltus and in Lygaeus are in agreement with the conclusions of Montgomery on Euschistus.

It will be apparent to all who read the description of the synaptic stages in the three Belostomatids contained in this paper that the same obscurities occur here as Wilson found in the Pentatomidae and that have so frequently been found in other organisms. This phenomenon can never be so thoroughly investigated in these forms as in those which do not suffer from these obscure stages. It seems very clear, however, that there is little or no evidence for telosynapsis in the Belostomatidae. The available evidence points decidedly toward parasynapsis as the prevailing mode in this family. Leptotene threads go into a synizetic knot and come out of this knot as thick threads of the haploid number. At first these pachytene threads show no indication of duality, but later a longitudinal line of separation typically occurs. There is no indication of an end-to-end union of chromatin threads at any stage, and hence the diplotene stage must be interpreted as a stage in parasynapsis at which the conjugating

threads separate somewhat from their more intimate previous relationship. As indicated in Lethocerus, the diplotene threads appear to open along the synaptic line and form the reticulum of the confused period by a branching and anastomosis of these threads. During the organization of the prophase chromatin elements there is additional evidence that parasynapsis is the mode of pairing. This is especially clear in the large ring-like tetrads of Lethocerus, but may also be justly regarded as in agreement with the observed facts in Belostoma and less clearly in Benacus. As already pointed out, the peculiarities of this last-named species makes it very difficult to reach a decision in regard to the matter in this animal.

As the matter stands at present, it appears to the author of this paper as very unlikely that telosynapsis occurs in any of the Hemiptera-Heteroptera. The only case of telosynapsis recently described in this group is that of Leptocoris trivittatus Say (Yocom, '23). Yocom has identified the youngest primary spermatocyte as "a small cell with a relatively large nucleus in which the chromatin is in the form of a much-tangled thread of a single strand lying in a clear space, quite apart from the nuclear membrane." Later, near the close of the growth period, the spireme undergoes a marked thickening and shortening and thus produces a rather typical synizetic knot. Yocom regards this as a stage corresponding to the true synizesis of other forms, but also concludes that this is not the period of synapsis as would be expected. Apparently this is followed immediately by the diffuse stage, so that it is impossible to determine the number of threads which emerge from the knot. Later, the chromatin is condensed into irregular masses which gradually become associated in pairs. Because these appear to be arranged in an end-to-end fashion, they are regarded as proving telosynapsis to be a fact in this animal. Added evidence is also seen in the fact that rings, double V's, double crosses, etc., so characteristic of forms with parasynapsis, do not occur in the period of tetrad formation in Leptocoris.

It would seem that the first mild contraction of the chromatin in Leptocoris corresponds to the first contraction of this material in Benacus which occurs at the same time. Following this stage in Benacus, there is some evidence of a split condition of the threads, whereas in Leptocoris there is no such evidence and no data on the number of threads present. It is quite possible that the conditions in Benacus may furnish a clew to the true condition in such forms as Leptocoris. The stages preceding the condition figured in Yocom's figure 3 should be carefully studied and also the stages immediately following, to determine, if possible, the further conditions at that time. While it is true, as contended by Yocom, that, in the light of our knowledge of synapsis in other groups, it is not too much to assume that both parasynapsis and telosynapsis may occur in the same family of bugs, nevertheless the frequency with which the discovery of this type of synapsis has been announced and later followed by a reversal of opinion tends to make one skeptical regarding its occurrence at all.

II. Chiasmatypie

Janssen's recent work ('24) on the spermatogenesis of Stethophyma grossum and Chorthippus parallelus has again brought the theory of chiasmatypie to the attention of cytologists. Apparently, these two Orthoptera are particularly favorable for studies of this nature and, as a result of his work on them, Janssens has brought forward even more striking evidence of a chiasma of chromatin threads than in the case of his earlier studies on Batrachia ('09, '19). Moreover, he has definitely answered the question so often asked regarding the exact time when these all-important chiasmas are established. In Stethophyma the leptotene threads form 'twin associations,' and while associated two by two in the leptotene stage they form the sutures which play such a large part in his theory. There may be only one suture, but frequently more than one occur, and there may be several. Later, there is a secondary splitting to form the four chromatids of the tetrads. When the tetrads are dis-

joined in the reduction division, the disjunction occurs in such a manner that segments of the chromatids are mutually exchanged, and thus the chromosomes delivered to the spermatids may come to possess segments previously belonging to its homologue.

Very little evidence of chiasmatypie has been obtained by other workers, even by those who have investigated the same group as Janssens. Gelei ('21, '22) has shown figures and described conditions in Dendrocoelum showing more or less interlacing among the chromatin threads. There is a possibility here of a regrouping of segments when condensation occurs. This particular condition appears to have been observed but rarely by Gelei, however. McClung ('24) has recently expressed himself as very definitely opposed to the theory, his opinion being that "there is absolutely no evidence that they (chromatin threads) break and recombine as Janssens' theory demands."[2]

At no stage in the maturation process is there any clear evidence of chiasmatypie in the Belostomatidae so far studied. It might be expected that such stages as represented in figures 58, 59, 76, 77, and similar figures should show chiasmas, if any exist. Careful examination of the chromatin threads at such stages do not reveal any signs of this peculiar relationship, so far as I have been able to determine. On the contrary, where the threads actually meet they merely seem to be closely approximated without twisting or interlacing. In such stages as represented in figure 77 the figure 8 is due simply to a distortion of the large ring tetrad as seen in profile view. There is here no actual twisting of one of the halves about the other. Indeed, the parts of the ring do not touch each other. As frequently pointed out by other workers, some sort of process of 'crossing over' must, doubtless, be accepted as proved by the large amount of genetical

[2] McClung ('27) has recently published the results of extensive work on Mecostethus grossus, the same species last studied by Janssens, and here reaffirms his denial of any process of chiasmatypie, such as the Belgian cytologist so carefully described.

data on the subject. Whether 'crossing over' occurs or not in the Belostomatidae is not known, but even if it were known, in lieu of evidence that a chiasmatypie occurs, we should remember that there are several obscure stages when it might occur. Possibly with improved technique the exact time of this interchange of segments of chromosomes may be discovered, and demonstrated by cytological means.

III. Origin and division of chondriosomes

As already stated, the study of chondriosomes in the Hemiptera-Heteroptera has been carried out by only one investigator (Bowen, '20, '22), according to the best methods now in use by cytologists.[3]

Montgomery ('11) attempted to solve the question of the origin and distribution of chondriosomes in Euschistus, but the technique upon which he relied is generally regarded as unsuited to such studies. He was not able to identify the chondriosomes with certainty in the spermatogonia, and this led him to look for their origination de novo in the cell. He concluded that they originate 'close to the idiosome' (which Bowen has shown does not exist) and the nucleus, and was thus led to regard them as arising from a joint action of idiosome and cytoplasm or of nucleus and cytoplasm. Furthermore, Montgomery suggested that the chondriosomes may be brought into formation in some manner in connection with the conjugation of the chromosomes, this being in keeping with his particular interpretation of the theory of the nucleus as a formative center. Shaffer ('20), working on Cicada septendecim (Homoptera), regarded the perinuclear position of the chondriosomes at the time they are increasing in amount as very important in shedding some light on the

[3] Since writing the above, Payne ('27) has published a piece of work on cytoplasmic structures in Gelastocoris oculatus (toad-bug), using a technique which makes it possible to follow these elements accurately. The chondriosomes are here first recognizable as two clusters in contact with the nuclear wall and in close physical relationship with a pair of chromosomes. Payne believes this indicates a close physiological relationship between chondriosomes and chromosomes.

question of their origin. He concluded that the evidence for the continuity of the chondriosomes has only a weak support. Although this author admits that a limited amount of the chondriosomal material may carry over from the previous mitosis to any given generation of cells, he nevertheless believes that these elements arise de novo "as differentiated parts of the cytoplasm through specific chemical (enzyme) reactions of the nucleus upon the products of assimilation of the cell."

Bowen ('20) has found the chondriosomes in the spermatogonia of the pentatomids in the form of granules and fine threads and has carefully followed them throughout the whole process of spermatogenesis. His work points toward the view that the chondriosomes are permanent cell organs.

The observations here recorded show that chondriosomes are present in the youngest spermatogonia and are continued in each cell generation throughout the successive mitoses in the germ cells until they are delivered to the spermatid in the form of a nebenkern. So far as this investigation goes, it must be regarded as favoring the view that chondriosomes are permanent cell organs, always present in plant and animal cells and derived by some sort of division process from preexisting elements of the same kind.

Wilson ('25) has recently examined this question of the permanency of chondriosomes within the cell and has expressed himself as rather favorable to the view that they are derived from preexisting ones and are not formed de novo from the cytoplasm.

The problem of the division of the chondriosomes is one which has attracted considerable attention among those cytologists who have turned their attention to cytoplasmic elements within recent years. Wilson ('16, '25 a, '25 b) has shown the mode of division of these bodies to be very precise in Centrurus where a large number of small chondriosomes fuse to form one large ring-like body. Subsequently, in the two maturation divisions, this ring divides equally in each division so that each spermatid receives exactly a fourth of

the total amount of chondriosomal material. The behavior in other scorpions is less striking and partakes more of a sorting-out process of the spherical chondriosomes into only approximately equal groups in the spermatids. Gatenby (Gatenby and Bhattacharya, '25) has recently announced the discovery of clear cases of division of these elements in late spermatogonial divisions of the Indian scorpion, Palamnaeus Bengalensis. The division appears to be of several types, such as binary fission, budding, etc. Voinov ('16, '25) has described an exceptionally clear and precise mode of division in the spermatocytes of Gryllotalpa vulgaris. In this animal the chondriosomes are spherical granules in the spermatogonia, but in the primary spermatocytes they become rods, and these fuse to form a skein which simulates the spireme of chromatin. Later, the skein fragments into a number of rods, and these become arranged in a palisade around the chromosomes in the equatorial plate and divide synchronously with them.

Conditions in the Hemiptera seem to be unfavorable for a study of the division of the chondriosomes if it occurs. It is entirely possible that the small, granular chondriosomes of the spermatogonia may divide and thus increase the number in each generation, but there is no satisfactory evidence of such a division. They can often be seen lying in positions suggesting division, but, on the other hand, this may be due to accidental placing. To settle this point, forms better suited for such study must be selected. Some isolated observations on another species of Lethocerus makes it seem possible to obtain some data on this matter from a study of that form.

IV. Golgi bodies

Bowen's ('20, '22) illuminating studies of these bodies in the pentatomids have shown that the so-called Golgi apparatus is extensively developed in the male germ cells in the form of numerous scattered plate-like bodies. These separate Golgi bodies are clearly made up of two substances, i.e.,

a deeply staining outer rim nearly surrounding a more lightly staining internal portion. During division of the spermatocytes the Golgi bodies fragment into a number of smaller dictyosomes, and these are distributed to the daughter cells and are finally aggregated and fused into single acroblasts in each of the spermatids. Bowen's theory that the central portion of each Golgi body is a part of the idiosome remains an interesting conjecture.

The present work on the Belostomatidae furnishes a rather striking confirmation of many of the observations of Bowen on the Pentatomidae with respect to the nature and behavior of the Golgi bodies. It appears to be very likely that all spermatogonia possess Golgi bodies, although they do not come into clear view until the growth period of the primary spermatocytes. From this time on until they fragment they can be accurately followed and their structure determined. They are practically identical in structure with those described by Bowen for the Pentatomidae. At about the time the nuclear membrane breaks down preparatory to the first division, the bodies fragment into a large number of small dictyosomes which are distributed to the daughter cells in both divisions. In this manner they are brought together in the spermatids, where they fuse to form the acroblast which eventually takes up a position in the young sperm at the anterior end of the cell.

SUMMARY AND CONCLUSIONS

Chromatin

1. The diploid number of chromosomes is twenty-eight in Benacus griseus, twenty-four in Belostoma flumineum, eight in Lethocerus americanus. There is no particular order in which the chromosomes occur in the nucleus nor in the equatorial plate. To some extent homologous pairs can be identified, but these do not become definitely associated in pairs until synapsis.

2. All three species possess an XY-pair of chromosomes. In the diploid state these can be positively identified only in Lethocerus. In Belostoma the X- and the Y-chromosomes differ considerably in size, but are usually of equal size in the other two species.

3. All spermatogonial divisions are equational and all chromosomes probably behave in like manner, except for the X- and the Y-chromosomes in Benacus, which show marked heteropycnosis in the later spermatogonial divisions.

4. Tetraploidy is frequently encountered in the follicular cells of the testis.

5. Probably in all three species the chromosomes come over from the last spermatogonial mitosis into the primary spermatocytes as leptotene threads without a stage of prochromosomes.

6. In Benacus the X- and Y-chromosomes are condensed precociously and are compact bodies clearly demonstrable throughout all succeeding stages. In Belostoma the condensation occurs at about the initiation of the leptotene stage, while in Lethocerus this does not occur until some time after synizesis. In all cases these elements are clearly visible at every stage subsequent to their original condensation.

7. In all three species the leptotene nuclei go over into a marked synizesis stage, which is also to be regarded as the stage of synapsis.

8. Following synizesis, there is evidence that the pachytene threads are in reality made up of two threads arranged side by side. Parasynapsis is therefore the mode of conjugation of homologous chromosomes in these Belostomatidae.

9. A confused period occurs in all three species. This period is particularly marked in Benacus, where there is a second contraction stage in the middle of the confused period.

10. The confused period appears to be brought on by a process of opening up and divergence of the paired elements followed by branching and anastomosis to form the characteristic reticulum of this period.

11. At the beginning of prophase the reticulum of the confused period is converted into tetrads. In Belostoma the tetrads are of several sorts, but their genesis and structure are not treated in this paper. In Lethocerus the three tetrads are all large atelomitic rings, whose structure is clearly shown. In Benacus the tetrads are of an obscure structure, but are probably modified paired rods.

12. Just before the final condensation of the tetrads in Benacus, another contraction phase occurs, during which all the chromatin elements are drawn(?) into the center of the nucleus. No such contraction occurs at this time in Lethocerus, but in Belostoma such a stage does occur, although in a characteristically different manner.

13. Fifteen separate chromosomes show in first maturation metaphases in Benacus, thirteen in Belostoma, and five in Lethocerus. Thus in every case the number of chromosomes at this stage is one more than the haploid number.

14. The first division is regarded as reductional for all autosomes and equational for the X- and Y-chromosomes.

15. In every case all chromosomes are divided in the first mitosis, with the result that each secondary spermatocyte receives one more than the haploid number.

16. The X- and the Y-chromosomes can be definitely identified at every step in the first division in Lethocerus, but not until later in the other two species.

17. During the telophase of the first division, the X- and the Y-chromosomes pair briefly and become arranged as dyads in the center of the second equatorial plate. At this time these chromosomes can be positively identified in all three species.

18. Polar views of secondary spermatocytes in metaphase in every case show exactly the haploid number of chromosomes.

19. In all three species the X- and the Y-chromosomes reduce during the second division, while the autosomes all divide equationally.

20. The result of this process is to deliver to each spermatid exactly the haploid number of chromosomes in all three species. In Benacus each spermatid receives fourteen chromosomes, in Belostoma each receives twelve, and in Lethocerus each gets four.

21. The dimorphism of the sperm with respect to the X- and the Y-chromosomes is perfectly clear in Belostoma, but is not so in Benacus and Lethocerus, because in these species these special chromosomes are usually of equal size.

The cytoplasmic inclusions

1. Chondriosomes are present in all spermatogonia as minute spherical granules. During the resting period they are usually massed close to the nuclear membrane. During mitosis they scatter throughout the cytoplasm and are apparently distributed equally to the daughter cells.

2. The chondriosomes become threads during the early growth period. In the two maturation divisions they are arranged in a palisade around the mitotic spindle and are roughly sorted out into approximately equal numbers in the daughter cells in both of these divisions.

3. Autonomous division of the chondriosomes has not been clearly observed. During anaphase and telophase of each division threads may be caught between the advancing cleavage furrow and cut in two.

4. In the spermatids the chondriosomes condense into a nebenkern, which shows many internal changes as it is being prepared to act as a sheath for the tail filament.

5. Golgi bodies are present in the spermatogonia as a number of separate minute granules.

6. No idiosome has been observed at any stage of the process of maturation in these animals.

7. The Golgi bodies grow rapidly during the early maturation stages. At the time of their maximum size, they show a very clear differentiation into two regions.

8. During the later prophases the Golgi bodies fragment into a large number of small dictyosomes, and these are dis-

tributed to the spermatids as a result of the two maturation divisions.

9. In the spermatids the Golgi bodies fuse to form an acroblast. This finally takes an anterior position in the sperm at about the time the nebenkern prepares to divide.

10. The centriole is present in spermatogonia as a single minute granule with no differentiated cytoplasm around it. It has not been traced through the resting period.

11. Following the last spermatogonial division, the centriole is lost to view during the period of synapsis and subsequent stages until the prophase, when it again appears as a double structure being already divided in preparation for both maturation divisions.

12. After the second division, the centrioles are again lost to view for some time, after which they reappear, probably double, and rapidly form a short tail filament. They then migrate to the base of the nucleus, where they remain.

13. The mitosome is a prominent structure in spermatogonia and, together with the midbody, forms a connecting element between neighboring cells of a cyst.

14. A mitosome is present following both maturation divisions, but neither it nor the associated midbody is a prominent feature of these divisions.

15. No important organ of the sperm is regarded as being derived from the mitosome.

16. Chromatoid bodies are present in the early spermatogonia and have been traced throughout all succeeding stages into the spermatids in which they are irregularly distributed.

17. Possibly there is one exception to the irregular behavior of the chromatoid bodies. In Belostoma, in many and possibly all of the spermatids, a granule, identified as a chromatoid body, accompanies the centriole in its movement to the base of the nucleus and sometimes obscures it.

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PLATES

EXPLANATION OF PLATES

All drawings have been first made in outline at table level with the camera lucida and, excepting figure 15, drawn with a 20× ocular and a 1.8-mm. apochromatic objective. Figure 15 was outlined with a 10× ocular and a 1.8-mm. apochromatic objective. In all cases the finer details have been drawn free-hand at a much lower magnification than used for outlining the drawings. All plates have been reduced one-fifth for publication. In all cases the method of preparation of the original material has been indicated. All figures are from Belostoma flumineum, Benacus griseus, and Lethocerus americanus, except figure 57, which is from an unidentified species of Lethocerus.

PLATE 1

EXPLANATION OF FIGURES

Figures 1, 13 to 19, and 23 are from Belostoma; figures 2, 3, 20 to 22, and 24 are from Benacus; figures 4 to 12 are from Lethocerus. Figures 1, 2, and 24 are from Flemming-hematoxylin material; figures 3 to 5, 7, 9, and 10 are from Bouin-hematoxylin material; figures 6, 8, 11 to 16, 18, and 20 to 23 are from modified Flemming-hematoxylin material; figure 19 is from Mann-Kopsch material.

1 Polar view of spermatogonial metaphase.

2 and 3 Polar views of spermatogonial metaphases.

4 to 6 Polar views of spermatogonial metaphases. The two smallest chromosomes are the XY-pair. Chondriosomes encircle the equatorial plate in 6.

7 and 8 Lateral views of spermatogonial metaphases. Chromatoid body at left in 7. Chondriosomes around spindle in 8 and remains of midbodies below.

9 and 10 Late spermatogonial anaphases. Chromosomes forming reticulum.

11 and 12 Resting spermatogonia showing lightly stained chondriosomes and deeply stained Golgi bodies. In 11, Golgi bodies are larger than usual, possibly from fusion. In 12, the whole mitosome shows clearly.

13 and 14 Resting spermatogonia with parts as before. In 13, the mitosome is sectioned transversely. In 14, the midbody is very prominent.

15 Section of a spermatogonial cyst, showing cells connected by mitosomes.

16 Resting spermatogonium, with chondriosomes arranged in a polar cap.

17 Resting spermatogonium, recently divided. Golgi bodies distinct.

18 Spermatogonial telophase. Chondriosomes spread through cytoplasm. Beginning of mitosome in the center.

19 Resting spermatogonium, with Golgi bodies heavily impregnated.

20 and 21 Spermatogonial prophases. Chondriosomes and Golgi bodies clearly shown.

22 Resting spermatogonium, with chondriosomes encircling nucleus; a few Golgi bodies at left and remains of mitosome above.

23 Resting tetraploid somatic cell, with massed chondriosomes and Golgi bodies.

24 Same as 23, with very large Golgi bodies and smaller agglutinated chondriosomes.

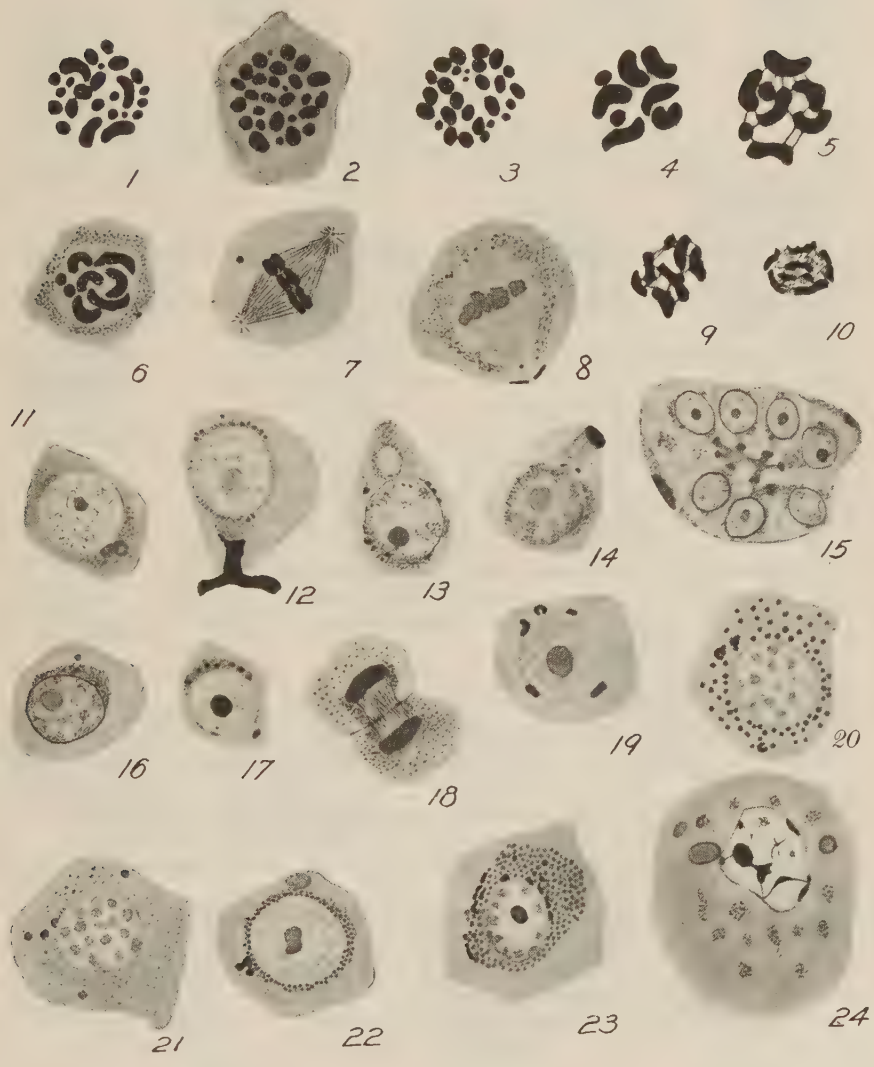
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PLATE 2

EXPLANATION OF FIGURES

Figures 25 to 32 and 44 to 47 are from Benacus; figures 33 to 38 and 40 to 43 are from Lethocerus; figure 39 is from Belostoma. Figures 25 to 27, 34 to 38, and 41 to 43 are from Bouin-hematoxylin material; figures 28 to 32, 39, and 44 to 47 are from Flemming-hematoxylin material; figures 33 and 40 are from Benda material.

25 Probably last spermatogonial anaphase; the two sex chromosomes more deeply stained than autosomes.

26 Probably last spermatogonial telophase; sex chromosomes as in 25; slight size difference.

27 Later telophase than 26.

28 Typical leptotene. XY-pair in contact in nuclear vacuole. Single Golgi body within mass of chondriosomes.

29 and 30 Typical synizesis. 29 shows only nucleus. Sex chromosomes distinct. Chondriosomes and Golgi bodies massed at nuclear pole.

31 Pachytene. Sex chromosomes in center. Partly destroyed chondriosomes spread through cytoplasm. Remains of Golgi body above.

32 Somewhat later pachytene. Parts as in 31.

33 Preleptotene with typical chondriosomes and Golgi bodies.

34 Preleptotene nucleus. Leptotene threads forming.

35 Leptotene nucleus. Occasional parallel arrangement of threads evident.

36 Synizesis beginning.

37 Later synizesis. Chromatoid body above.

38 Nucleus showing extreme contraction of chromatin in synizesis.

39 Synizesis. Chondriosomes and Golgi bodies at left.

40 and 41 Synizesis. Chondriosomes and Golgi bodies clear in 40. Nucleus only in 41.

42 Emergence from synizesis. Plasmosome large. XY-pair condensing.

43 Typical pachytene nucleus. Plasmosome large. XY-pair not shown.

44 Late pachytene. XY-pair clear. Remains of chondriosomes and Golgi bodies at right.

45 Still later pachytene, showing double nature of some of the threads. Nucleus only. Plasmosome in center.

46 Confused stage beginning. XY-pair still compact. Remains of chondriosomes and Golgi bodies surrounding nucleus.

47 Later confused stage. Other parts as in 46.

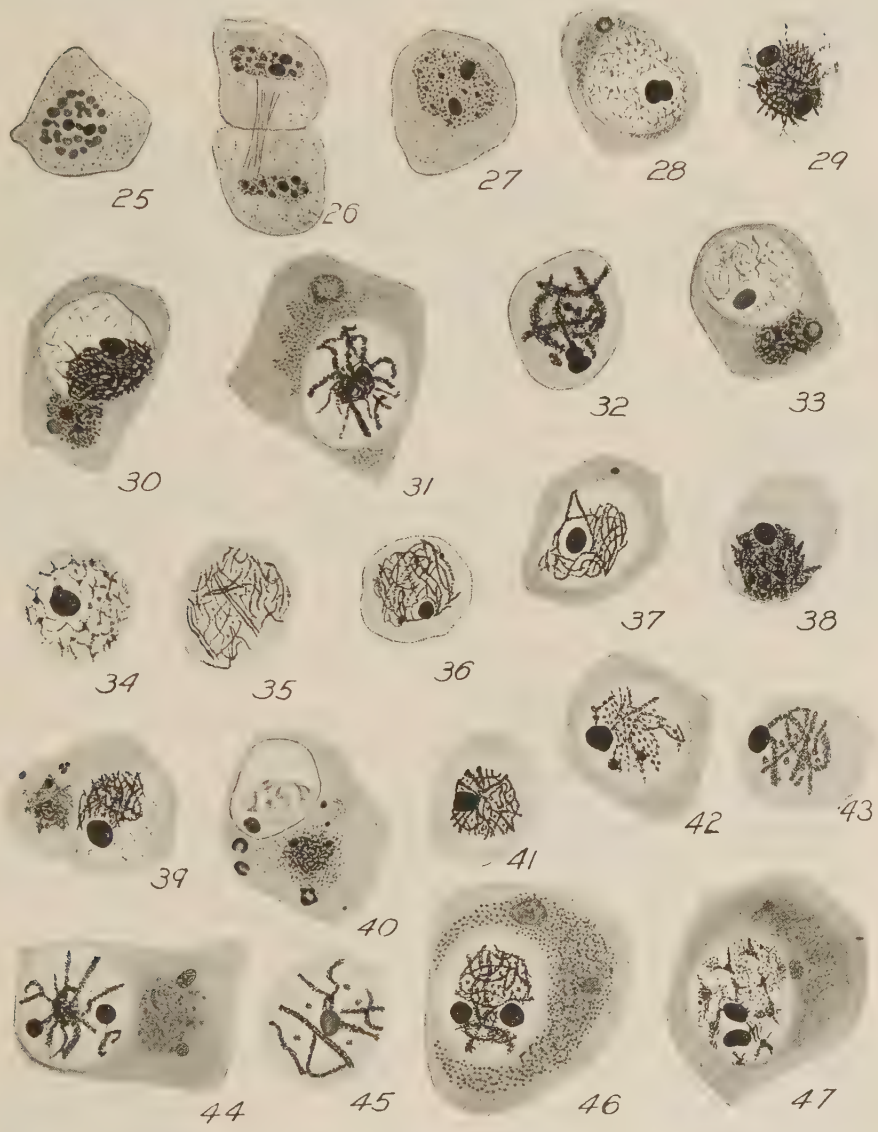
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PLATE 3

EXPLANATION OF FIGURES

Figures 48 to 53 and 65 to 71 are from Benacus; figures 62 to 64 are from Belostoma; figures 54 to 61 are from Lethocerus. Figures 48 to 51, 53, 57, 63 to 69, and 71 are from Flemming-hematoxylin material; figures 52, 54, 55, and 70 are from Benda material; figures 56, 58 to 60, and 62 are from Bouin-hematoxylin material; figure 61 is from Mann-Kopsch material.

48 and 49 Nuclei during height of first confused stage. XY-pair clear.

50 Nucleus during second contraction stage. XY-pair compact and deeply staining.

51 Later second contraction. Remains of chondriosomes and Golgi bodies around the nucleus. Two small chromatoid bodies, above, right and left.

52 Nearly same stage as 51. Golgi bodies at their clearest. Chondriosomes forming threads from granules.

53 Tetrads beginning to condense. XY-pair in center. Chondriosomes and Golgi bodies as in 52, but injured by technique.

54 and 55 Pachytene stages. Chondriosomes and Golgi bodies typical.

56 and 57 Diplotene nuclei. One whole thread is shown in 57; this is from an unidentified species.

58 Diplotene nucleus going into confused stage. XY-pair above. Plasmosome below.

59 Portion of a double thread. Same stage as 58.

60 Nucleus at height of confused stage. XY-pair now condensed.

61 Group of selected Golgi bodies.

62 Confused stage. Plasmosome, left center; XY-pair in contact, right center.

63 Selected Golgi bodies from abnormal individual.

64 Selected Golgi bodies.

65 Condensation of tetrads beginning. XY-pair in center. Chromatin masses around periphery of nucleus. Chondriosomes and Golgi bodies as before.

66 Tetrads well developed. Nucleus only.

67 Tetrads further condensed, showing paired condition. Cytoplasm contains chromatoid bodies, centrioles, Golgi bodies, and chondriosomes.

68 Three large tetrads, showing centers of condensation.

69 Group of tetrads, showing variety of form just before final steps in condensation.

70 Tetrad stage with chondriosomes and Golgi bodies intermixed.

71 Contraction of tetrads just before nuclear membrane breaks down. Nucleus only.

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PLATE 4

EXPLANATION OF FIGURES

Figures 72 to 74 and 89 to 91 are from Benacus; figures 75 to 83 are from Lethocerus; figures 84 to 88 are from Belostoma. Figures 72 to 74, 77, 78, 89, and 90 are from Flemming-hematoxylin material; figures 75, 76, 80, 81, and 83 are from Bouin-hematoxylin material; figures 79, 82, and 91 are from Benda material; figures 84, 85, and 91 are from modified Flemming-hematoxylin material; figures 86 and 88 are from Mann-Kopsch material.

72 to 74 Three stages in the formation of the first spindle. Chromatoid bodies numerous. Chondriosomes and Golgi bodies altered by technique.

75 and 76 Two nuclei, showing stages in condensation of ring tetrads. XY-pair in 76.

77 Ring tetrad twisted into a figure 8.

78 A series of tetrads, showing progressive condensation of a single ring to form a bivalent chromosome.

79 Tetrad stage to show chondriosomes and Golgi bodies.

80 Typical primary spermatocyte. Three chromatoid bodies.

81 Bivalent chromosomes, end view, when nuclear membrane breaks down.

82 Stage similar to 80, from a cyst containing smaller cells with prominent chondriosomes and Golgi bodies.

83 Tetrads going upon the spindle partly open.

84 Late prophase. Chondriosomes and Golgi bodies very clear.

85 Later prophase. Chondriosomes somewhat broken. Golgi bodies granular.

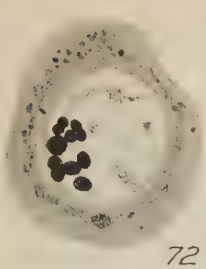
86 Late prophase, just as nuclear membrane breaks down. Golgi bodies fragmenting.

87 Equatorial plate forming. Golgi bodies fragmented.

88 Nearly same stage as 87, showing dictyosomes grouped.

89 and 90 Polar views of first spermatocyte metaphases. The small chromosome at right in 89 and at left in 90 is easily mistaken for a chromatoid body, above in 90.

91 Same as 89 and 90. Chondriosomes typical. One chromosome missing; probably three chondriosomes within chromosome group.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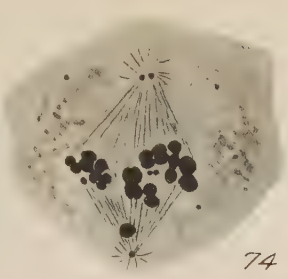
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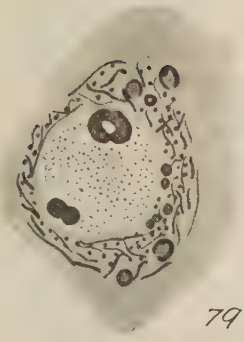
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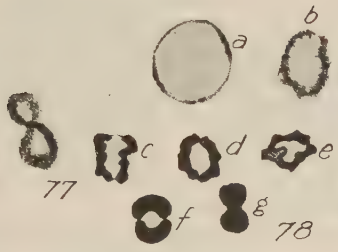
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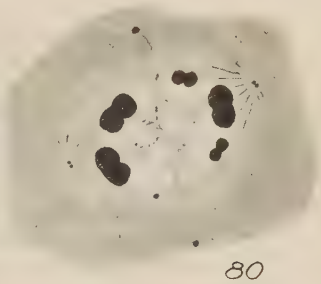
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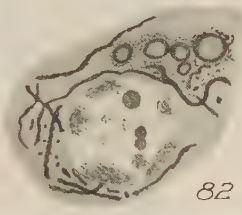
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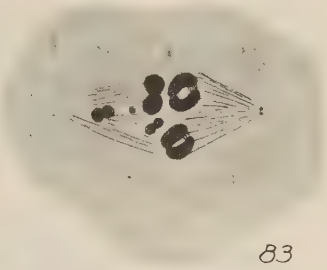
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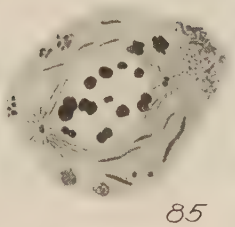
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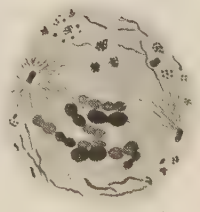
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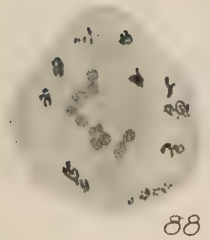
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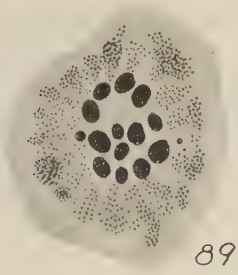
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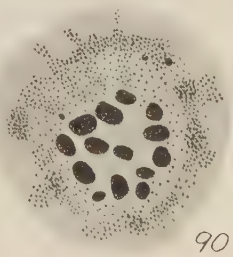
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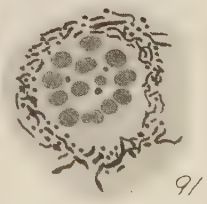
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PLATE 5

EXPLANATION OF FIGURES

Figures 92 to 95 and 102 to 107 are from Benacus; figures 96 to 101 are from Belostoma; figures 108 to 110 are from Lethocerus. Figures 92, 95, 99, 100 to 102, 104, 105, 107 to 109 are from Flemming-hematoxylin material; figures 93, 94, 103, 106, and 110 are from Benda material; figure 96 is from modified Flemming-hematoxylin material; figure 97 is from Bouin-hematoxylin material; figure 98 is from Mann-Kopsch material.

92 First maturation metaphase. Chromatoid body at right.

93 Same as 92; chondriosomes typical.

94 Surface view of primary spermatocyte, showing full length of chondriosomes.

95 Anaphase group of chromosomes, first division. Chondriosomes reduced to a granular mass.

96 Metaphase of first division; polar view. Chondriosomes agglutinated.

97 Same as 96. Chromatoid bodies in the cytoplasm.

98 Same stage as 96 and 97. Dictyosomes grouped as produced by fragmentation of Golgi bodies.

99 and 100 - Metaphase and anaphase of first division. Chondriosomes partly destroyed by technique.

101 First division, polar view of early anaphase chromosome group. Probably some of the dictyosomes showing among the chondriosomal material.

102 Late anaphase of first division.

103 Telophase of first division.

104 Slightly later than 103. Midbody beginning at right. Large chromatoid body at left.

105 Second metaphase, polar view.

106 Second metaphase, lateral view.

107 Second metaphase. XY-pair in center. Three chromatoid bodies.

108 First metaphase, polar view. XY-pair are the two smallest. Single chromatoid body to right. Chondriosomes as before.

109 First metaphase. Parts as in 108.

110 First metaphase. Chondriosomes very clear for this species.

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PLATE 6

EXPLANATION OF FIGURES

Figures 111 to 121 are from Lethocerus; figures 122 to 125 are from Benacus; figures 126 to 129 are from Belostoma. Figures 111 to 113, 115 to 117, 119, and 120 are from Bouin-hematoxylin material; figures 114, 118, 121, and 125 are from Benda material; figures 122 to 124 are from Flemming-hematoxylin material; figures 126, 128, and 129 are from modified Flemming-hematoxylin material; figure 127 is from Mann-Kopsch material.

111 Early anaphase of first division. Chromatoid bodies large.

112 Late anaphase of first division. One autosome missing at right. Large chromatoid body in center, unusually placed.

113 Polar view of first-division anaphase. Autosomes show split for second division.

114 Late anaphase of first division.

115 Polar view of anaphase chromosomes in same stage as 114.

116 Second metaphase, polar view.

117 Second metaphase. XY-pair at the right.

118 Second metaphase. Chondriosomes typical.

119 Rather late second anaphase. XY-pair separated.

120 Polar view of anaphase chromosomes in same stage as 119.

121 Same as 119. Chondriosomes typical.

122 Typical second anaphase.

123 and 124 Second anaphase chromosomes, polar view.

125 Late anaphase of second division. Chondriosomes typical.

126 First-division telophase. Chondriosomes agglutinated.

127 First telophase. Dictyosomes passing into the secondary spermatocytes.

128 Second metaphase, polar view. Chondriosomes peripherally placed.

129 Second metaphase. XY-pair in the center. Chondriosomes as in 128.

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PLATE 7

EXPLANATION OF FIGURES

Figures 130 to 134, 144 to 146, 151, and 153 are from Belostoma; figures 135 to 141 and 147 to 150 are from Benacus; figures 142, 143, and 152 are from Lethocerus. Figures 130, 135, 136, 138 to 141, 148 to 150, and 152 are from Benda material; figures 132, 133, 144, 146, 151, and 153 are from modified Flemming-hematoxylin material; figure 134 is from Mann-Kopsch material; figures 131, 137, 145, and 147 are from Flemming-hematoxylin material; figures 142 and 143 are from Bouin-hematoxylin material.

130 to 133 Second anaphase stages. Two kinds of spermatids shown in 131 and 132; either X or Y in the circle of autosomes. In 133 the chondriosomes are typical for this species. Chromatoid body just to right of chromosomes in spermatid to the right.

134 Late anaphase of second division. Dictyosomes passing into the spermatids.

135 to 140 Various stages of second telophase. Typical position of chondriosomes in early spermatids in 135. In 136 some chondriosomes are divided transversely. In 137 chondriosomes are shown following ordinary technique. 138 and 139 are polar views of two closely successive stages in the very early condensation of chondriosomes to form the nebenkern. In 140 groups of chondriosomes are shown from the side; just a little later than 139.

141 Further condensation of the nebenkern; somewhat later stage.

142 and 143 Late second telophase in 142, showing lengthened spindle fibers. 143 shows a spermatid with four chromosomes still distinct within newly built nucleus. Remains of nebenkern at left. Chromatoid body in both figures, below.

144 to 146 Second telophases. In 144 dictyosomes show as deeply stained granules among masses of chondriosomes; small midbody in the center. 145 is same stage as 144. 146 is a polar view of later stage with nebenkern condensing around the nucleus.

147 to 150 Spermatids. 147 shows vacuolated nebenkern above, nucleus below with two centrioles on its right and acroblast and chromatoid body on its left. 148 shows several Golgi bodies around the nebenkern. A vacuole, below, is a common structure at this stage. In 149 centriole, below nucleus, is rod-like. Nebenkern is developing chromophobic and chromophilic portions. Acroblast is typical. Centriole has grown an axial filament in 150.

151 to 153 Spermatid and young sperm. Parts as before. Two chromatoid bodies are between nebenkern and acroblast in 151. In 152 centriole is migrating to base of nucleus. Acroblast and nebenkern below. Centriole always appears to be accompanied by a chromatoid body in this species, shown in 153.

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